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(54) Title: ISOLATED NUCLEIC ACID MOLECULES ENCODING CANCER ASSOCIATED ANTIGENS, THE ANTIGENS PER SE, AND USES THEREOF

(57) Abstract: The invention relates to newly identified cancer associated antigens. It has been discovered that each of these molecules provokes antibodies when expressed by a subject. The ramifications of this observation are also a part of this invention.

ISOLATED NUCLEIC ACID MOLECULES ENCODING CANCER ASSOCIATED
ANTIGENS, THE ANTIGENS PER SE, AND USES THEREOF

RELATED APPLICATIONS

This application is a continuation in part of Serial No. 09/602, 362, filed June 22, 2000 which is a continuation in part of Serial No. 09/451,739, filed November 30, 1999, both of which are incorporated by reference in their entirety.

FIELD OF THE INVENTION

This invention relates to antigens associated with cancer, the nucleic acid molecules encoding them, as well as the uses of these.

BACKGROUND AND PRIOR ART

It is fairly well established that many pathological conditions, such as infections, cancer, autoimmune disorders, etc., are characterized by the inappropriate expression of certain molecules. These molecules thus serve as "markers" for a particular pathological or abnormal condition. Apart from their use as diagnostic "targets", i.e., materials to be identified to diagnose these abnormal conditions, the molecules serve as reagents which can be used to generate diagnostic and/or therapeutic agents. A by no means limiting example of this is the use of cancer markers to produce antibodies specific to a particular marker. Yet another non-limiting example is the use of a peptide which complexes with an MHC molecule, to generate cytolytic T cells against abnormal cells.

Preparation of such materials, of course, presupposes a source of the reagents used to generate these. Purification from cells is one laborious, far from sure method of doing so.

Another preferred method is the isolation of nucleic acid molecules which encode a particular marker, followed by the use of the isolated encoding molecule to express the desired molecule.

Two basic strategies have been employed for the detection of such antigens, in e.g., human tumors. These will be referred to as the genetic approach and the biochemical approach. The genetic approach is exemplified by, e.g., dePlaen et al., Proc. Natl. Sci. USA 85: 2275 (1988), incorporated by reference. In this approach, several hundred pools of plasmids of a cDNA library obtained from a tumor are transfected into recipient cells, such as COS cells, or into antigen-negative variants of tumor cell lines which are tested for the expression of the specific antigen. The biochemical approach, exemplified by, e.g., O. Mandelboim, et al., Nature 369: 69 (1994) incorporated by reference, is based on acidic elution of peptides which have bound to MHC-class I molecules of tumor cells, followed by reversed-phase high performance liquid chromatography (HPLC). Antigenic peptides are identified after they bind to empty MHC-class I molecules of mutant cell lines, defective in antigen processing, and induce specific reactions with cytotoxic T-lymphocytes. These reactions include induction of CTL proliferation, TNF release, and lysis of target cells, measurable in an MTT assay, or a ⁵¹Cr release assay.

These two approaches to the molecular definition of antigens have the following disadvantages: first, they are enormously cumbersome, time-consuming and expensive; and second, they depend on the establishment of cytotoxic T cell lines (CTLs) with predefined specificity.

The problems inherent to the two known approaches for the identification and molecular definition of antigens is best demonstrated by the fact that both methods have, so far, succeeded in defining only very few new antigens in human tumors. See, e.g., van der Bruggen et al., Science 254: 1643-1647 (1991); Brichard et al., J. Exp. Med. 178: 489-495 (1993); Coulie, et

al., J. Exp. Med. 180: 35-42 (1994); Kawakami, et al., Proc. Natl. Acad. Sci. USA 91: 3515-3519 (1994).

Further, the methodologies described rely on the availability of established, permanent cell lines of the cancer type under consideration. It is very difficult to establish cell lines from certain cancer types, as is shown by, e.g., Oettgen, et al., Immunol. Allerg. Clin. North. Am. 10: 607-637 (1990). It is also known that some epithelial cell type cancers are poorly susceptible to CTLs in vitro, precluding routine analysis. These problems have stimulated the art to develop additional methodologies for identifying cancer associated antigens.

One key methodology is described by Sahin, et al., Proc. Natl. Acad. Sci. USA 92: 11810-11913 (1995), incorporated by reference. Also, see U.S. Patent No. 5,698,396, and Application Serial No. 08/479,328, filed on June 7, 1995 and January 3, 1996, respectively. All three of these references are incorporated by reference. To summarize, the method involves the expression of cDNA libraries in a prokaryotic host. (The libraries are secured from a tumor sample). The expressed libraries are then immunoscreened with absorbed and diluted sera, in order to detect those antigens which elicit high titer humoral responses. This methodology is known as the SEREX method ("Serological identification of antigens by Recombinant Expression Cloning"). The methodology has been employed to confirm expression of previously identified tumor associated antigens, as well as to detect new ones. See the above referenced patent applications and Sahin, et al., supra, as well as Crew, et al., EMBO J 144: 2333-2340 (1995).

This methodology has been applied to a range of tumor types, including those described by Sahin et al., supra, and Pfreundschuh, supra, as well as to esophageal cancer (Chen et al., Proc. Natl. Acad. Sci. USA 94: 1914-1918 (1997)); lung cancer (Güre et al., Cancer Res. 58:

1034-1041 (1998)); colon cancer (Serial No. 08/948, 705 filed October 10, 1997) incorporated by reference, and so forth. Among the antigens identified via SEREX are the SSX2 molecule (Sahin et al., Proc. Natl. Acad. Sci. USA 92: 11810-11813 (1995); Tureci et al., Cancer Res. 56: 4766-4772 (1996); NY-ESO-1 Chen, et al., Proc. Natl. Acad. Sci. USA 94: 1914-1918 (1997); and SCP1 (Serial No. 08/892,705 filed July 15, 1997) incorporated by reference. Analysis of SEREX identified antigens has shown overlap between SEREX defined and CTL defined antigens. MAGE-1, tyrosinase, and NY-ESO-1 have all been shown to be recognized by patient antibodies as well as CTLs, showing that humoral and cell mediated responses do act in concert.

It is clear from this summary that identification of relevant antigens via SEREX is a desirable aim. The inventors have applied this methodology and have identified several new antigens associated with cancer, as detailed in the description which follows.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

EXAMPLE 1

The SEREX methodology, as described by, e.g. Sahin, et al., Proc. Natl. Acad. Sci. USA 92: 11810-11813 (1995); Chen, et al., Proc. Natl. Acad. Sci. USA 94: 1914-1918 (1997), and U.S. Patent No. 5,698,396, all of which are incorporated by reference. In brief, total RNA was extracted from a sample of a cutaneous metastasis of a breast cancer patient (referred to as "BR11" hereafter), using standard CsCl guanidine thiocyanate gradient methodologies. A cDNA library was then prepared, using commercially available kits designed for this purpose. Following the SEREX methodology referred to supra, this cDNA expression library was amplified, and screened with either autologous BR11 serum which had been diluted to 1:200, or with allogeneic, pooled serum, obtained from 7 different breast cancer patients, which had been

diluted to 1:1000. To carry out the screen, serum samples were first diluted to 1:10, and then preabsorbed with lysates of *E. coli* that had been transfected with naked vector, and the serum samples were then diluted to the levels described supra. The final dilutions were incubated overnight at room temperature with nitrocellulose membranes containing phage plaques, at a density of 4-5000 plaque forming units ("pfus") per 130 mm plate.

Nitrocellulose filters were washed, and incubated with alkaline phosphatase conjugated, goat anti-human Fc γ secondary antibodies, and reactive phage plaques were visualized via incubation with 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium.

This procedure was also carried out on a normal testicular cDNA library, using a 1:200 serum dilution.

A total of 1.12×10^6 pfus were screened in the breast cancer cDNA library, and 38 positive clones were identified. With respect to the testicular library, 4×10^5 pfus were screened, and 28 positive clones were identified.

Additionally, 8×10^5 pfus from the BR11 cDNA library were screened using the pooled serum described. Of these, 23 were positive.

The positive clones were subcloned, purified, and excised to forms suitable for insertion in plasmids. Following amplification of the plasmids, DNA inserts were evaluated via restriction mapping (EcoRI-XbaI), and clones which represented different cDNA inserts were sequenced using standard methodologies.

If sequences were identical to sequences found in GenBank, they were classified as known genes, while sequences which shared identity only with ESTs, or were identical to nothing in these data bases, were designated as unknown genes. Of the clones from the breast cancer library which were positive with autologous serum, 3 were unknown genes. Of the

remaining 35, 15 were identical to either NY-ESO-1, or SSX2, two known members of the CT antigen family described supra, while the remaining clones corresponded to 14 known genes. Of the testicular library, 12 of the clones were SSX2.

The NY-ESO-1 antigen was not found, probably because the commercial library that was used had been size fractionated to have an average length of 1.5 kilobases, which is larger than full length NY-ESO-1 cDNA which is about 750 base pairs long.

With respect to the screening carried out with pooled, allogeneic sera, four of the clones were NY-ESO-1. No other CT antigens were identified. With the exception of NY-ESO-1, all of the genes identified were expressed universally in normal tissue.

A full listing of the isolated genes, and their frequency of occurrence follows, in tables 1, 2 and 3. Two genes were found in both the BR11 and testicular libraries, i.e., poly (ADP-ribose) polymerase, and tumor suppression gene ING1. The poly (ADP-ribose) polymerase gene has also been found in colon cancer libraries screened via SEREX, as is disclosed by Scanlan, et al., Int. J. Cancer 76: 652-58 (1998) when the genes identified in the screening of the BR11 cDNA library by autologous and allogeneic sera were compared, NY-ESO-1 and human keratin.

Table 1. SEREX-defined genes identified by autologous screening of BR11 cDNA library

Gene group	No. of clones	Comments	Expression
CT genes	10	NY-ESO-1	tumor, testis
	5	SSX2	tumor, testis
Non-CT genes	5	Nuclear Receptor Co-Repressor	ubiquitous
	4	Poly(ADP-ribose) polymerase	ubiquitous
	2	Adenylosuccinatelyase	ubiquitous
	2	cosmid 313 (human)	ESTs: muscle, brain, breast
	1	CD 151 (transmembrane protein)	ubiquitous

1	Human HRY Gen	RT-PCR: multiple normal tissues
1	Alanyl-t-RNA-Synthetase	ubiquitous
1	NAD(+) ADP-Ribosyltransferase	ubiquitous
1	Human keratin 10	ESTs: multiple normal tissues
1	Human EGFR kinase substrate	ubiquitous
1	<i>ING 1</i> Tumor suppressor gene	RT-PCR: multiple normal tissues
1	Unknown gene, NCI_CGAP_Pr12 cDNA clone	ESTs: pancreas, liver, spleen, uterus
1	Unknown gene	ESTs: multiple normal tissues
1	Unknown gene	RT-PCR: multiple normal tissues

Table 2. SEREX-defined genes identified by allogeneic screening of BR11 cDNA library

Gene group	No. of clones	Comments	Expression
CT genes	4	NY-ESO-1	tumor, testis
Non-CT genes	6	zinc-finger helicase	ESTs: brain, fetal heart, total fetus
	4	Acetoacetyl-CoA-thiolase	ubiquitous
	3	KIAA0330 gene	ESTs: multiple normal tissues
	2	U1snRNP	ubiquitous
	1	Human aldolase A	ubiquitous
	1	Retinoblastoma binding protein 6	ESTs: tonsils, fetal brain, endothelial cells, brain
	1	α 2-Macroglobulin receptor associated protein	ubiquitous
	1	Human Keratin 10	ESTs: multiple normal tissues

Table 3. SEREX-defined genes identified by screening of a testicular cDNA library with BR11 serum

Gene group	No. of clones	Comments	Expression
CT genes:	12	SSX2	tumor, testis
Non-CT genes:	3	Rho-associated coiled-coil forming protein	ubiquitous
	3	Poly(ADP-ribose) polymerase	ubiquitous

3	Gene from HeLa cell, similar to TITIN	ubiquitous
2	Gene from parathyroid tumor	RT-PCR: multiple normal tissues
1	Transcription termination factor I-interacting peptide 21	ubiquitous
1	Gene from fetal heart	ESTs: multiple normal tissues
1	<i>ING 1</i> tumor suppressor gene	RT-PCR: multiple normal tissues
1	KIAA0647 cDNA	ESTs: multiple normal tissues
1	KIAA0667 cDNA	ESTs: multiple normal tissues

EXAMPLE 2

The mRNA expression pattern of the cDNAs identified in example 1, in both normal and malignant tissues, was studied. To do this, gene specific oligonucleotide primers were designed which would amplify cDNA segments 300-600 base pairs in length, using a primer melting temperature of 65-70° C. The primers used for amplifying MAGE-1,2,3 and 4, BAGE, NY-ESO-1, SCP1, and SSX1, 2, 3, 4 and 5 were known primers, or were based on published sequences. See Chen, et al. supra; Tureci, et al., Proc. Natl. Acad. Sci. USA 95: 5211-16 (1998). Gure, et al., Int. J. Cancer 72: 965-71 (1997); Chen, et al., Proc. Natl. Acad. Sci. USA 91: 1004-1008 (1994); Gaugler, et al., J. Exp. Med. 179: 921-930 (1994), dePlaen, et al., Immunogenetics 40: 360-369 (1994), all of which are incorporated by reference. RT-PCR was carried out for 35 amplification cycles, at an annealing temperature of 60° C. Using this RT-PCR assay, the breast cancer tumor specimen was positive for a broad range of CT antigens, including MAGE-1,3 AND 4, BAGE, SSX2, NY-ESO-1 and CT7. The known CT antigens SCP-1, SSX1, 4 and 5 were not found to be expressed.

An additional set of experiments were carried out, in which the seroreactivity of patient sera against tumor antigens was tested. Specially, ELISAs were carried out, in accordance with

Stockert, et al., J. Exp. Med. 187: 1349-1354 (1998), incorporated by reference, to determine if antibodies were present in the patient sera. Assays were run for MAGE-1, MAGE-3, NY-ESO-1, and SSX2. The ELISAs were positive for NY-ESO-1 and SSX2, but not the two MAGE antigens.

EXAMPLE 3

Two clones (one from the breast cancer cDNA library and one from the testicular library), were identified as a gene referred to as ING1, which is a tumor suppressor gene candidate. See Garkavtsev, et al., Nature 391: 295-8 (1998), incorporated by reference. The sequence found in the breast cancer library, differed from the known sequence of ING1 at six residues, i.e., positions 818, 836, 855, 861, 866 and 874. The sequence with the six variants is set forth at SEQ ID NO: 1. The sequence of wild type ING1 is set out at SEQ ID NO: 2.

To determine if any of these differences represented a mutation in tumors, a short, PCR fragment which contained the six positions referred to supra was amplified from a panel of allogeneic normal tissue, subcloned, amplified, and sequenced following standard methods.

The results indicated that the sequences in the allogeneic tissues were identical to what was found in tumors, ruling out the hypothesis that the sequence differences were a tumor associated mutation. This conclusion was confirmed, using the testicular library clone, and using restriction analysis of ING1 cDNA taken from normal tissues. One must conclude, therefore, that the sequence information provided by Garkavtsev, et al., supra, is correct.

EXAMPLE 4

Additional experiments were carried out to determine whether genetic variations might exist in the 5' portion of the ING1 gene, which might differ from the 5' portion of the clone discussed supra (SEQ ID NO: 1). In a first group of experiments, attempts were made to obtain full length ING1 cDNA from both the breast tumor library, and the testicular library. SEQ ID NO: 1 was used as a probe of the library, using standard methods.

Four clones were isolated from the testicular library and none were isolated from the breast cancer library. The four clones, following sequencing, were found to derive from three transcript variants. The three variants were identical from position 586 down to their 3' end, but differed in their 5' regions, suggesting alternatively spliced variants, involving the same exon-intron junction. All three differed from the sequence of ING1 described by Garkavtsev, et al., in Nat. Genet. 14: 415-420 (1996). These three variants are set out as SEQ ID NOS: 1, 3 and 4.

All of the sequences were then analyzed. The ORFs of SEQ ID NOS: 2, 1 and 4 (SEQ ID NO: 2 is the originally disclosed, ING1 sequence), encode polypeptides of 294, 279 and 235 amino acids, of which 233 are encoded by the 3' region common to the three sequences. These putative sequences are set out as SEQ ID NOS: 19, 5, and 7. With respect to SEQ ID NO: 3, however, no translational initiation site could be identified in its 5' region.

EXAMPLE 5

The data regarding SEQ ID NO: 3, described supra, suggested further experiments to find additional ORFs in the 5'-end of variant transcripts of the molecule. In order to determine this, 5'-RACE-PCR was carried out using gene specific and adapted specific primers, together with commercially available products, and standard methodologies.

The primers used for these experiments were:

CACACAGGATCCATGTTGAGTCCTGCCAACGG

CGTGGTCGTGGTTGCTGGACGCG

(SEQ ID NOS: 9 and 10), for SEQ ID NO: 1;

CCCAGCGGCCCTGACGCTGTC

CGTGGTCGTGGTTGCTGGACGCG

(SEQ ID NOS: 11 and 12), for SEQ ID NO: 3; and

GGAAGAGATAAGGCCTAGGGAAG

CGTGGTCGTGGTTGCTGGACGCG

(SEQ ID NOS: 13 and 14), for SEQ ID NO: 4.

Cloning and sequencing of the products of RACE PCR showed that the variant sequence of SEQ ID NO: 4 was 5' to SEQ ID NO: 3, and that full length cDNA for the variant SEQ ID NO: 3 contained an additional exon 609 nucleotides long, positioned between SEQ ID NO: 3 and the shared, 3' sequence referred to supra. This exon did not include an ORF. The first available initiation site would be an initial methionine at amino acid 70 of SEQ ID NO: 1. Thus, if expressed, SEQ ID NO: 3 would correspond to a molecule with a 681 base pair, untranslated 5' end and a region encoding 210 amino acids (SEQ ID NO:6).

EXAMPLE 6

The presence of transcript variants with at least 3 different transcriptional initiation sites, and possibly different promoters, suggested that mRNA expression might be under different, tissue specific regulation.

To determine this, variant-specific primers were synthesized, and RT-PCR was carried out on a panel of tissues, using standard methods.

SEQ ID NO: 1 was found to be expressed universally in all of the normal breast, brain and testis tissues examined, in six breast cancer lines, and 8 melanoma cell lines, and in cultured melanocytes. SEQ ID NO: 3 was found to be expressed in four of the six breast cancer lines, normal testis, liver, kidney, colon and brain. SEQ ID NO: 4 was only found to be expressed by normal testis cells and weakly in brain cells.

EXAMPLE 7

A further set of experiments were carried out to determine if antibodies against ING1 were present in sera of normal and cancer patients. A phase plaque immuno assay of the type described supra was carried out, using clones of SEQ ID NO: 1 as target. Of 14 allogeneic sera taken from breast cancer patients, two were positive at 1:200 dilutions. All normal sera were negative.

EXAMPLE 8

The BR11 cDNA library described supra was then screened, using SEQ ID NO: 1 and standard methodologies. A 593 base pair cDNA was identified, which was different from any sequences in the data banks consulted. The sequence of this cDNA molecule is set out at SEQ ID NO: 8.

The cDNA molecule set forth as SEQ ID NO:1 was then used in Southern blotting experiments. In brief, genomic DNA was isolated from normal human tissue, digested with BamHI or Hind III, and then separated onto 0.7% agarose gel, blotted onto nitrocellulose filters,

and hybridized using ^{32}P labelled SEQ ID NO: 1, at high stringency conditions (aqueous buffer, 65 °C). The probes were permitted to hybridize overnight, and then exposed for autoradiography. Two hybridizing DNA species were identified, i.e., SEQ ID NOS: 1 and 8.

EXAMPLE 9

The cDNA molecule set forth in SEQ ID NO: 8 was then analyzed. 5'- RACE PCR was carried out using normal fetus cDNA. Full length cDNA for the molecule is 771 base pairs long, without the poly A tail. It shows strong homology to SEQ ID NO: 1, with the strongest homology in the 5' two-thirds (76% identity over nucleotide 1-480); however, the longest ORF is only 129 base pairs, and would encode a poly peptide 42 amino acids long which was homologous to, but much shorter than, the expected expression product of SEQ ID NO: 1.

In addition to the coding region, SEQ ID NO: 8 contains 203 base pairs of 5'-untranslated region, and 439 base pairs of 3'-untranslated region.

RT-PCR assays were carried out, as described supra. All of the normal tissues tested, including brain, colon, testis, tissue and breast, were positive for expression of this gene. Eight melanoma cell lines were tested, of which seven showed varying levels of expression, and one showed no expression. Six breast cancer cell lines were tested, of which four showed various levels of expression, and two showed no expression.

EXAMPLE 10

An additional breast cancer cDNA library, referred to as "BR17-128", was screened, using autologous sera. A cDNA molecule was identified.

Analysis of the sequence suggested that it was incomplete at the 5' end. To extend the sequence, a testicular cDNA library was screened with a nucleotide probe based upon the partial sequence identified in the breast cancer library. An additional 1200 base pairs were identified following these screenings. The 2011 base pairs of information are set forth in SEQ ID NO: 15.

The longest open reading frame is 1539 base pairs, corresponding to a protein of about 59.15 kilodaltons. The deduced sequence is set forth at SEQ ID NO: 16.

RT-PCR was then carried out using the following primers:

CACACAGGATCCATGCAGGCCCGCACAAAGGAG

CACACAAAGCTTCTAGGATTTGGCACAGCCAGAG

(SEQ ID NOS: 17 and 18)

Strong signals were observed in normal testis and breast tissue, and weak expression was observed in placenta.

No expression was found in normal brain, kidney, liver, colon, adrenal, fetal brain, lung, pancreas, prostate, thymus, uterus, and ovary tissue of tumor cell lines tested, 2 of the breast cancer lines were strongly positive and two were weakly positive. Of melanoma two of 8 were strongly positive, and 3 were weakly positive. Of lung cancer cell lines, 4 of 15 were strongly positive, and 3 were weakly positive.

When cancer tissue specimens were tested, 16 of 25 breast cancer samples were strongly positive, and 3 additional samples were weakly positive. Two of 36 melanoma samples were positive (one strong, one weak). All other cancer tissue samples were negative.

When Northern blotting was carried out, a high molecular weight smear was observed in testis, but in no other tissues tested.

EXAMPLE 11

Further experiments were carried out using the tumor sample referred to in example 10, supra. This sample was derived from a subcutaneous metastasis of a 60 year old female breast cancer patient. Total RNA was extracted, as described supra. Following the extraction, a cDNA library was constructed in λ -ZAP expression vectors, also as described supra. Screening was carried out, using the protocol set forth in example 1. A total of 7×10^5 pfus were screened. Fourteen reactive clones were identified, purified, and sequenced. The sequences were then compared to published sequences in GenBank and EST databases. These analyses indicated that the clones were derived from seven distinct genes, two of which were known, and five unknown. The two known genes were "PBK-1" (three clones), and TI-227 (one clone). These are universally expressed genes, with the libraries referred to supra showing ESTs for these genes from many different tissues.

With respect to the remaining 10 clones, six were derived from the same gene, referred to hereafter as "NY-BR-1." Three cDNA sequences were found in the EST database which shared identity with the gene. Two of these (AI 951118 and AW 373574) were identified as being derived from a breast cancer library, while the third (AW 170035), was from a pooled tissue source.

EXAMPLE 12

The distribution of the new gene NY-BR-1 referred to supra was determined via RT-PCR. In brief, gene specific oligonucleotide NY-BR-1 primers were designed to amplify cDNA

segments 300-600 base pairs in length, with primer melting temperatures estimated at 65-70°C.

The RT-PCR was then carried out over 30 amplification cycles, using a thermal cycler, and an annealing temperature of 60°C. Products were analyzed via 1.5% gel electrophoresis, and ethidium bromide visualization. Fifteen normal tissues (adrenal gland, fetal brain, lung, mammary gland, pancreas, placenta, prostate, thymus, uterus, ovary, brain, kidney, liver, colon and testis) were assayed. The NY-BR-1 clone gave a strong signal in mammary gland and testis tissue, and a very faint signal in placenta. All other tissues were negative. The other clones were expressed universally, based upon comparison to information in the EST database library, and were not pursued further.

The expression pattern of NY-BR-1 in cancer samples was then tested, by carrying out RT-PCR, as described supra, on tumor samples.

In order to determine the expression pattern, primers:

caaagcagag cctcccgaga ag

(SEQ ID NO: 20) and

cctatgctgc tcttcgattc ttcc

(SEQ ID NO: 21) were used.

Of twenty-five breast cancer samples tested, twenty two were positive for NY-BR-1. Of these, seventeen gave strong signals, and five gave weak to modest signals.

An additional 82 non-mammary tumor samples were also analyzed, divided into 36 melanoma, 26 non small cell lung cancer, 6 colon cancer, 6 squamous cell carcinoma, 6 transitional cell carcinoma, and two leiomyosarcomas. Only two melanoma samples were positive for NY-BR-1 expression.

The study was then extended to expression of NY-BR-1 in tissue culture. Cell lines derived from breast tumor, melanoma, and small cell lung cancer were studied. Four of six breast cancer cells were positive (two were very weak), four of eight melanoma (two very weak), and seven of fourteen small cell lung cancer lines (two very weak) were positive.

EXAMPLE 13

In order to determine the complete cDNA molecule for NY-BR-1, the sequences of the six clones referred to supra were compiled, to produce a nucleotide sequence 1464 base pairs long. Analysis of the open reading frame showed a continuous ORF throughout, indicating that the compiled sequence is not complete.

Comparison of the compiled sequence with the three EST library sequences referred to supra allowed for extension of the sequence. The EST entry AW170035 (446 base pairs long) overlapped the compiled sequence by 89 base pairs at its 5' end, permitting extension of the sequence by another 357 base pairs. A translational terminal codon was identified in this way, leading to a molecule with a 3'-untranslated region 333 base pairs long. The 5' end of the molecule was lacking, however, which led to the experiment described infra.

EXAMPLE 14

In order to determine the missing, 5' end of the clone described supra, a commercially available testis cDNA expression library was screened, using a PCR expression product of the type described supra as a probe. In brief, 5×10^4 pfus per 150 mm plate were transferred to nitrocellulose membranes, which were then submerged in denaturation solution (1.5M NaCl and 0.5 M NaOH), transferred to neutralization solution (1.5 M NaCl and 0.5M Tris-HCl), and then

rinsed with 0.2M Tris-HCl, and 2xSSC. Probes were labelled with ^{32}P and hybridization was carried out at high stringency conditions (i.e., 68°C, aqueous buffer). Any positive clones were subcloned, purified, and in vivo excised to plasmid PBK-CMV, as described supra.

One of the clones identified in this way included an additional 1346 base pairs at the 5' end; however, it was not a full length molecule. A 5'-RACE-PCR was carried out, using commercially available products. The PCR product was cloned into plasmid vector pGEMT and sequenced. The results indicated that cDNA sequence was extended 1292 base pairs further, but no translation initiation site could be determined, because no stop codons could be detected. It could be concluded, however, that the cDNA of the NY-BR17 clone comprises at least 4026 nucleotides, which are presented as SEQ ID NO: 22. The molecule, as depicted, encodes a protein at least about 152.8 kDA in molecular weight. Structurally, there are 99 base pairs 5' to the presumed translation initiation site, and an untranslated segment 333 base pairs long at the 3' end. The predicted amino acid sequence of the coding region for SEQ ID NO: 22 is set out at SEQ. ID NO: 23.

SEQ ID NO: 23 was analyzed for motifs, using the known search programs PROSITE and Pfam. A bipartite nuclear localization signal motif was identified at amino acids 17-34, suggesting that the protein is a nuclear protein. Five tandem ankyrin repeats were identified, at amino acids 49-81, 82-114, 115-147, 148-180 and 181-213. A bZIP site (i.e. a DNA binding site followed by a leucine zipper motif) was found at amino acid positions 1077-1104, suggesting a transcription factor function. It was also observed that three repetitive elements were identified in between the ankyrin repeats and the bZIP DNA binding site. To elaborate, a repetitive element 117 nucleotides long is trandemly repeated 3 times, between amino acids 459-815. The second repetitive sequence, consisting of 11 amino acids, repeats 7 times between amino acids

224 and 300. The third repetitive element, 34 amino acids long, is repeated twice, between amino acids 301-368.

EXAMPLE 15

The six clones described supra were compared, and analysis revealed that they were derived from two different splice variants. Specifically, two clones, referred to as "BR17-8" and "BR 17-44a", contain one more exon, of 111 base pairs (nucleotides 3015-3125 of SEQ ID NO: 22), which encodes amino acids 973-1009 of SEQ ID NO: 23, than do clones BR 17-1a, BR17-35b and BR17-44b. The shortest of the six clones, BR17-128, starts 3' to the additional exons. The key structural elements referred to supra were present in both splice variants, suggesting that there was no difference in biological function.

The expression pattern of the two splice variants was assessed via PT-PCR, using primers which spanned the 111 base pair exon referred to supra.

The primers used were:

aatggaaca	agagctctgc	ag
(SEQ ID NO: 24) and		
gggtcatctg	aagttcagca	ttc
(SEQ ID NO: 25)		

Both variants were expressed strongly in normal testis and breast. The longer variant was dominant in testis, and the shorter variant in breast cells. When breast cancer cells were tested, co-typing of the variant was observed, (7 strongly, 2 weakly positive, and 1 negative), with the shorter variant being the predominant form consistently.

EXAMPLE 16

The frequency of antibody response against NY-BR-1 in breast cancer patients was tested. To do this, a recombinant protein consisting of amino acids 993-1188 of SEQ ID NO: 23 was prepared. (This is the protein encoded by clone BR 17-128, referred to supra). A total of 140 serum samples were taken from breast cancer patients, as were 60 normal serum samples. These were analyzed via Western blotting, using standard methods.

Four of the cancer sera samples were positive, including a sample from patient BR17. All normal sera were negative.

An additional set of experiments was then carried out to determine if sera recognized the portion of NY-BR-1 protein with repetitive elements. To do this, a different recombinant protein, consisting of amino acids 405-1000 was made, and tested in Western blot assays. None of the four antibody positive sera reacted with this protein indicating that an antibody epitope is located in the non-repetitive, carboxy terminal end of the molecule.

EXAMPLE 17

The screening of the testicular cDNA library referred to supra resulted, inter alia, in the identification of a cDNA molecule that was homologous to NY-BR-1. The molecule is 3673 base pairs in length, excluding the poly A tail. This corresponded to nucleotides 1-3481 of SEQ ID NO: 22, and showed 62% homology thereto. No sequence identity to sequences in libraries was noted. ORF analysis identified an ORF from nucleotide 641 through the end of the sequence, with 54% homology to the protein sequence of SEQ. ID NO: 23. The ATG initiation codon of this sequence is 292 base pairs further 3' to the presumed initiation codon of NY-BR-1, and is preceded by 640 untranslated base pairs at its 5' end. This 640 base pair sequence includes

scattered stop codons. The nucleotide sequence and deduced amino acid sequence are presented as SEQ ID NOS: 26 and 27, respectively.

RT-PCR analysis was carried out in the same way as is described supra, using primers:

tct catagat gctggtgctg atc

(SEQ ID NO: 28) and

cccagacatt gaattttggc agac

(SEQ ID NO: 29).

Tissue restricted mRNA expression was found. The expression pattern differed from that of SEQ ID NO: 22. In brief, of six normal tissues examined, strong signals were found in brain and testis only. There was no or weak expression in normal breast tissues, and kidney, liver and colon tissues were negative. Eight of ten 10 breast cancer specimens tested supra were positive for SEQ. ID NO: 26. Six samples were positive for both SEQ. ID NO: 22 and 26, one for SEQ. ID NO: 22 only, two for the SEQ. ID NO: 26 only, and one was negative for both.

EXAMPLE 18

Recently, a working draft of the human genome sequence was released. This database was searched, using standard methods, and NY-BR-1 was found to have sequence identity with at least three chromosome 10 clones, identified by Genbank accession numbers AL157387, AL37148, and AC067744. These localize NY-BR-1 to chromosome 10 p11.21-12.1.

The comparison of NY-BR-1 and the human genomic sequence led to definition of NY-BR-1 exon-intron organization. In brief, the coding region of the gene contains essentially 19 structurally distinct exons with at least 2 exons encoding 3' untranslated regions. Detailed exon-intron junction information is described at Genbank AF 269081.

The six ankyrin repeats, referred to supra, are all found within exon 7. The 357 nucleotide repeating unit is composed of exons 10-15. The available genomic sequences are not complete, however, and only one of the three copies was identified, suggesting that DNA sequences between exons 5 and 10 may be duplicated and inserted in tandem, during genetic evolution. In brief, when the isolated NY-BR-1 cDNA clone was analyzed, three complete and one incomplete copy of the repeating units are present. The exon sequences can be expressed as exons 1-2-3-4-5-6-7-8-9-(10-11-12-13-14-15)-(10A-11A-12A-13A-14A-15A)-(10B-11B-12B-13B-14B-15B)-(10C-11C-12C-13C-14C)-16-17-18-19-20-21, wherein A, B & C are inexact copies of exon 10-15 sequences. Cloned, NY-BR-1 cDNA has 38 exons in toto.

It was noted, supra, that the sequence of NY-BR-1 cDNA was not complete at the 5' end. Genomic sequence (Genbank AC067744), permitted extension of the 5' end. Translation of the 5' genomic sequence led to the identification of a new translation initiation site, 168 base pairs upstream of the previously predicted ATG initiation codon. This led to an NY-BR-1 polypeptide including 1397 amino acid longer, 56 residue of which are added at the N-terminus, compared to prior sequence information, i.e.:

MEEISAAAVKVVPGERPSPFSQLVYTSNDSYIVHSGDLRKIHKAASRGQVRKLEK (SEQ ID NO: 30).

EXAMPLE 20

Reference was made, supra, to the two difference splice variants of NY-BR-1. Comparison of the splice variants with the genomic sequence confirmed that an alternate splicing event, with the longer variant incorporating part of intron 33 into exon 34 (i.e., exon 17 of the basic exon/intron framework described supra).

Key structural elements that were predicted in NY-BR-1, described supra, are present in both variants, suggesting that there is no difference in biological function, or subcellular location.

EXAMPLE 21

As with NY BR-1, the variant NY-BR-1.1, described supra, was screened against the working draft of the human genome sequence. One clone was found with sequence identity, i.e., GenBank AL359312, derive from chromosome 9. Thus, NY-BR-1 and NY-BR-1.1 both appear to be functioning genes, on two different chromosomes. The Genbank sequence referred to herein does not contain all of NY-BR-1.1, which precludes defining exon-intron structure. Nonetheless, at least 3 exons can be defined, which correspond to exons 16-18 of the NY-BR-1 basic framework. Exon-intron junctions are conserved.

EXAMPLE 22

A series of peptides were synthesized, based upon the amino acid sequence of NY-BR-1, as set forth in SEQ ID NO: 23. These were then tested for their ability to bind to HLA-A2 molecules and to stimulate CTL proliferation, using an ELISPOT assay. This assay involved coating 96-well, flat bottom nitrocellulose plates with 5ug/ml of anti-interferon gamma antibodies in 100 ul of PBS per well, followed by overnight incubation. Purified CD8⁺ cells, which had been separated from PBL samples via magnetic beads coated with anti-CD8 antibodies were then added, at 1x10⁵ cells/well, in RPMI 1640 medium, that had been supplemented with 10% human serum, L-asparagine (50 mg/l), L-arginine (242 mg/l), L-glutamine (300 mg/l), together with IL-2 (2.5ng/ml), in a final volume of 100 ul. CD8⁺ effector

cells were prepared by presensitizing with peptide, and were then added at from 5×10^3 to 2×10^4 cells/well. Peptides were pulsed onto irradiated T2 cells at a concentration of 10ug/ml for 1 hour, washed and added to effector cells, at 5×10^4 cells/well. The plates were incubated for 16 hours at 37°C, washed six times with 0.05% Tween 20/PBS, and were then supplemented with biotinylated, anti-interferon gamma specific antibody at 0.5 ug/ml. After incubation for 2 hours at 37°C, plates were washed, and developed with commercially available reagents, for 1 hour, followed by 10 minutes of incubation with dye substrate. Plates were then prepped for counting, positives being indicated by blue spots. The number of blue spots/well was determined as the frequency of NY-ESO-1 specific CTLs/well.

Experiments were run, in triplicate, and total number of CTLs was calculated. As controls, one of reagents alone, effector cells alone, or antigen presenting cells alone were used. The difference between the number of positives in stimulated versus non-stimulated cells, was calculated as the effective number of peptide specific CTLs above background. Three peptides were found to be reactive, i.e.:

LLSHGAVIEV (amino acids 102-111 of SEQ ID NO: 23)

SLSKILDTV (amino acids 904-912 of SEQ ID NO: 23)

SLDQKLFQL (amino acids 1262-1270 of SEQ ID NO: 23).

The complete list of peptides tested, with reference to their position in SEQ ID NO: 23, follows:

Peptide	Position
FLVDRKVCQL	35-43
ILIDSGADI	68-76

AVYSEILSV	90-98
ILSVVAKLL	95-103
LLSHGAVIEV	102-111
KLLSHGAVI	101-109
FLLIKNANA	134-142
MLLQQNVDV	167-175
GMLLQQNVDV	166-175
LLQQNVDVFA	168-177
IAWEKKETPV	361-370
SLFESSAKI	430-438
CIPENSIYQKV	441-450
KVMEINREV	449-457
ELMDMQTFKA	687-696
ELMDMQTFKA	806-815
SLSKILDTV	904-912
KILDTVHSC	907-915
ILNEKIREEL	987-996
RIQDIELKSV	1018-1027
YLLHENCML	1043-1051
CMLKKEIAML	1049-1058
AMLKLELATL	1056-1065
KILKEKNAEL	1081-1090
VLIAENTML	1114-1122
CLQRKMNV DV	1174-1183
KMNV DV SST	1178-1186
SLDQKLFQL	1262-1270
KLFQLQSKNM	1266-1275

FQLQSKNMWL	1268-1277
QLQSKNMWL	1269-1277
NMWLQQQLV	1274-1282
WLQQQLVHA	1276-1284
KITIDIHFL	1293-1301

The foregoing examples describe the isolation of a nucleic acid molecule which encodes a cancer associated antigen. "Associated" is used herein because while it is clear that the relevant molecule was expressed by several types of cancer, other cancers, not screened herein, may also express the antigen.

The invention relates to nucleic acid molecules which encode the antigens encoded by, e.g., SEQ ID NOS: 1, 3, 8, 15, 22 and 26 as well as the antigens encoded thereby, such as the proteins with the amino acid sequences of SEQ ID NOS: 5, 6, 7, 16, 23, 27, and 30. It is to be understood that all sequences which encode the recited antigen are a part of the invention.

Also a part of the invention are proteins, polypeptides, and peptides, which comprise, e.g., at least nine consecutive amino acids found in SEQ ID NO: 23, or at least nine consecutive amino acids of the amino acids of SEQ ID NO: 30. Proteins, polypeptides and peptides comprising nine or more amino acids of SEQ ID NO: 5, 6, 7, 16 or 27 are also a part of the invention. Especially preferred are peptides comprising or consisting of amino acids 102-111, 904-912, or 1262-1270 of SEQ ID NO: 23. Such peptides may, but do not necessarily provoke CTL responses when complexed with an HLA molecule, such as an HLA-A2 molecule. They may also bind to different MHC or HLA molecules, including, but not being limited to, HLA-A1, A2, A3, B7, B8, Cw3, Cw6, or serve, e.g., as immunogens, as part of immunogenic cocktail compositions, where they are combined with other proteins or polypeptides, and so forth. Also

a part of the invention are the nucleic acid molecules which encode these molecules, such as "minigenes," expression vectors that include the coding regions, recombinant cells containing these, and so forth. All are a part of the invention.

Also a part of the invention are expression vectors which incorporate the nucleic acid molecules of the invention, in operable linkage (i.e., "operably linked") to a promoter. Construction of such vectors, such as viral (e.g., adenovirus or Vaccinia virus) or attenuated viral vectors is well within the skill of the art, as is the transformation or transfection of cells, to produce eukaryotic cell lines, or prokaryotic cell strains which encode the molecule of interest. Exemplary of the host cells which can be employed in this fashion are COS cells, CHO cells, yeast cells, insect cells (e.g., Spodoptera frugiperda), NIH 3T3 cells, and so forth. Prokaryotic cells, such as E. coli and other bacteria may also be used. Any of these cells can also be transformed or transfected with further nucleic acid molecules, such as those encoding cytokines, e.g., interleukins such as IL-2, 4, 6, or 12 or HLA or MHC molecules.

Also a part of the invention are the antigens described herein, both in original form and in any different post translational modified forms. The molecules are large enough to be antigenic without any posttranslational modification, and hence are useful as immunogens, when combined with an adjuvant (or without it), in both precursor and post-translationally modified forms. Antibodies produced using these antigens, both poly and monoclonal, are also a part of the invention as well as hybridomas which make monoclonal antibodies to the antigens. The whole protein can be used therapeutically, or in portions, as discussed infra. Also a part of the invention are antibodies against this antigen, be these polyclonal, monoclonal, reactive fragments, such as Fab, (F(ab)₂)' and other fragments, as well as chimeras, humanized antibodies, recombinantly produced antibodies, and so forth.


As is clear from the disclosure, one may use the proteins and nucleic acid molecules of the invention diagnostically. The SEREX methodology discussed herein is premised on an immune response to a pathology associated antigen. Hence, one may assay for the relevant pathology via, e.g., testing a body fluid sample of a subject, such as serum, for reactivity with the antigen per se. Reactivity would be deemed indicative of possible presence of the pathology. So, too, could one assay for the expression of any of the antigens via any of the standard nucleic acid hybridization assays which are well known to the art, and need not be elaborated upon herein. One could assay for antibodies against the subject molecules, using standard immunoassays as well.

Analysis of SEQ ID NO: 1, 3, 4, 8, 15, 22 and 26 will show that there are 5' and 3' non-coding regions presented therein. The invention relates to those isolated nucleic acid molecules which contain at least the coding segment, and which may contain any or all of the non-coding 5' and 3' portions.

Also a part of the invention are portions of the relevant nucleic acid molecules which can be used, for example, as oligonucleotide primers and/or probes, such as one or more of SEQ ID NOS: 9, 10, 11, 12, 13, 14, 17, 18, 20, 21, 24, 25, 28, and 29 as well as amplification products like nucleic acid molecules comprising at least nucleotides 305-748 of SEQ ID NO: 1, or amplification products described in the examples, including those in examples 12, 14, etc.

As was discussed supra, study of other members of the "CT" family reveals that these are also processed to peptides which provoke lysis by cytolytic T cells. There has been a great deal of work on motifs for various MHC or HLA molecules, which is applicable here. Hence, a further aspect of the invention is a therapeutic method, wherein one or more peptides derived from the antigens of the invention which bind to an HLA molecule on the surface of a patient's

tumor cells are administered to the patient, in an amount sufficient for the peptides to bind to the MHC/HLA molecules, and provoke lysis by T cells. Any combination of peptides may be used. These peptides, which may be used alone or in combination, as well as the entire protein or immunoreactive portions thereof, may be administered to a subject in need thereof, using any of the standard types of administration, such as intravenous, intradermal, subcutaneous, oral, rectal, and transdermal administration. Standard pharmaceutical carriers, adjuvants, such as saponins, GM-CSF, and interleukins and so forth may also be used. Further, these peptides and proteins may be formulated into vaccines with the listed material, as may dendritic cells, or other cells which present relevant MHC/peptide complexes.

Similarly, the invention contemplates therapies wherein nucleic acid molecules which encode the proteins of the invention, one or more or peptides which are derived from these proteins are incorporated into a vector, such as a Vaccinia or adenovirus based vector, to render it transfectable into eukaryotic cells, such as human cells. Similarly, nucleic acid molecules which encode one or more of the peptides may be incorporated into these vectors, which are then the major constituent of nucleic acid bases therapies. 

Any of these assays can also be used in progression/regression studies. One can monitor the course of abnormality involving expression of these antigens simply by monitoring levels of the protein, its expression, antibodies against it and so forth using any or all of the methods set forth supra.

It should be clear that these methodologies may also be used to track the efficacy of a therapeutic regime. Essentially, one can take a baseline value for a protein of interest using any of the assays discussed supra, administer a given therapeutic agent, and then monitor levels of the protein thereafter, observing changes in antigen levels as indicia of the efficacy of the regime.

As was indicated supra, the invention involves, inter alia, the recognition of an "integrated" immune response to the molecules of the invention. One ramification of this is the ability to monitor the course of cancer therapy. In this method, which is a part of the invention, a subject in need of the therapy receives a vaccination of a type described herein. Such a vaccination results, e.g., in a T cell response against cells presenting HLA/peptide complexes on their cells. The response also includes an antibody response, possibly a result of the release of antibody provoking proteins via the lysis of cells by the T cells. Hence, one can monitor the effect of a vaccine, by monitoring an antibody response. As is indicated, supra, an increase in antibody titer may be taken as an indicia of progress with a vaccine, and vice versa. Hence, a further aspect of the invention is a method for monitoring efficacy of a vaccine, following administration thereof, by determining levels of antibodies in the subject which are specific for the vaccine itself, or a large molecule of which the vaccine is a part.

The identification of the subject proteins as being implicated in pathological conditions such as cancer also suggests a number of therapeutic approaches in addition to those discussed supra. The experiments set forth supra establish that antibodies are produced in response to expression of the protein. Hence, a further embodiment of the invention is the treatment of conditions which are characterized by aberrant or abnormal levels of one or more of the proteins, via administration of antibodies, such as humanized antibodies, antibody fragments, and so forth. These may be tagged or labelled with appropriate cystostatic or cytotoxic reagents.

T cells may also be administered. It is to be noted that the T cells may be elicited in vitro using immune responsive cells such as dendritic cells, lymphocytes, or any other immune responsive cells, and then reperfused into the subject being treated.

Note that the generation of T cells and/or antibodies can also be accomplished by administering cells, preferably treated to be rendered non-proliferative, which present relevant T cell or B cell epitopes for response, such as the epitopes discussed supra.

The therapeutic approaches may also include antisense therapies, wherein an antisense molecule, preferably from 10 to 100 nucleotides in length, is administered to the subject either "neat" or in a carrier, such as a liposome, to facilitate incorporation into a cell, followed by inhibition of expression of the protein. Such antisense sequences may also be incorporated into appropriate vaccines, such as in viral vectors (e.g., Vaccinia), bacterial constructs, such as variants of the known BCG vaccine, and so forth.

Other features and applications of the invention will be clear to the skilled artisan, and need not be set forth herein. The terms and expression which have been employed are used as terms of description and not of limitation, and there is no intention in the use of such terms and expression of excluding any equivalents of the features shown and described or portions thereof, it being recognized that various modifications are possible within the scope of the invention.

We claim:

1. Isolated nucleic acid molecule which encodes a cancer associated antigen, whose amino acid sequence is identical to the amino acid sequence encoded by the nucleotide sequence of SEQ ID NO: 1, 3, 4, 8, 15, 19, 22, or 26.
2. The isolated nucleic acid molecule of claim 1, comprising the nucleotide sequence of SEQ ID NO: 1.
3. The isolated nucleic acid molecule of claim 1, comprising the nucleotide sequence of SEQ ID NO: 3.
4. The isolated nucleic acid molecule of claim 1, comprising the nucleotide sequence of SEQ ID NO: 4.
5. The isolated nucleic acid molecule of claim 1, comprising the nucleotide sequence of SEQ ID NO: 8.
6. The isolated nucleic acid molecule of claim 1, comprising the nucleotide sequence of SEQ ID NO: 15.
7. The isolated nucleic acid molecule of claim 1, comprising the nucleotide sequence of SEQ ID NO: 19.
8. The isolated nucleic acid molecule of claim 1, comprising the nucleotide sequence of SEQ ID NO: 22.
9. The isolated nucleic acid molecule of claim 1, comprising the nucleotide sequence of SEQ ID NO: 26.
10. Expression vector comprising the isolated nucleic acid molecule of claim 1, operably linked to a promoter.

11. Eukaryotic cell line or prokaryotic cell strain, transformed or transfected with the expression vector of claim 10.
12. Isolated cancer associated antigen comprising all or part of the amino acid sequence encoded by SEQ ID NO: 1, 3, 4, 8, 15, 19, 22 or 26.
13. Eukaryotic cell line or prokaryotic cell strain, transformed or transfected with the isolated nucleic acid molecule of claim 1.
14. The eukaryotic cell line or prokaryotic cell strain of claim 13, wherein said cell line is also transfected with a nucleic acid molecule coding for a cytokine.
15. The eukaryotic cell line or prokaryotic cell strain of claim 14, wherein said cell line is further transfected by a nucleic acid molecule coding for an MHC molecule.
16. The eukaryotic cell line or prokaryotic cell strain of claim 14, wherein said cytokine is an interleukin.
17. The eukaryotic cell line or prokaryotic cell strain of claim 16, wherein said interleukin is IL-2, IL-4 or IL-12.
18. The eukaryotic cell line or prokaryotic cell strain of claim 13, wherein said cell line has been rendered non-proliferative.
19. The eukaryotic cell line of claim 13, wherein said cell line is a fibroblast cell line.
20. Expression vector comprising a mutated or attenuated virus and the isolated nucleic acid molecule of claim 1.
21. The expression vector of claim 20, wherein said virus is adenovirus or vaccinia virus.
22. The expression vector of claim 21, wherein said virus is vaccinia virus.
23. The expression vector of claim 21, wherein said virus is adenovirus.

24. Expression system useful in transfecting a cell, comprising (i) a first vector containing a nucleic acid molecule which codes for the isolated cancer associated antigen of claim 13 and (ii) a second vector selected from the group consisting of (a) a vector containing a nucleic acid molecule which codes for an MHC or HLA molecule which presents an antigen derived from said cancer associated antigen and (b) a vector containing a nucleic acid molecule which codes for an interleukin.

25. Immunogenic composition comprising the isolated cancer antigen of claim 12, and a pharmaceutically acceptable adjuvant.

26. The immunogenic composition of claim 25, wherein said adjuvant is a cytokine, a saponin, or GM-CSF.

27. Immunogenic composition comprising at least one peptide consisting of an amino acid sequence of from 8 to 12 amino acids concatenated to each other in the isolated cancer associated cancer antigen of claim 12, and a pharmaceutically acceptable adjuvant.

28. The immunogenic composition of claim 27, wherein said adjuvant is a saponin, a cytokine, or GM-CSF.

29. The immunogenic composition of claim 25, wherein said composition comprises a plurality of peptides which complex with a specific MHC molecule.

30. Immunogenic composition which comprises at least one expression vector which encodes a peptide derived from the amino acid sequence encoded by SEQ ID NO: 1, 3, 4, 8, 15, 19, 22 or 26.

31. The immunogenic composition of claim 30, wherein said at least one expression vector codes for a plurality of peptides.

32. Vaccine useful in treating a subject afflicted with a cancerous condition comprising the isolated eukaryotic cell line of claim 13 and a pharmacologically acceptable adjuvant.

33. The vaccine of claim 32, wherein said eukaryotic cell line has been rendered non-proliferative.

34. The vaccine of claim 33, wherein said eukaryotic cell line is a human cell line.

35. A composition of matter useful in treating a cancerous condition comprising a non-proliferative cell line having expressed on its surface a peptide derived from the amino acid sequence encoded by SEQ ID NO: 1, 3, 4, 8, 15, 19, 22 or 26.

36. The composition of matter of claim 35, wherein said cell line is a human cell line.

37. A composition of matter useful in treating a cancerous condition, comprising (i) a peptide derived from the amino acid sequence encoded by SEQ ID NO: 1, 3, 4, 8, 15, 19, 22 or 26, (ii) an MHC or HLA molecule, and (iii) a pharmaceutically acceptable carrier.

38. Isolated antibody which is specific for the cancer associated antigen of claim 12.

39. The isolated antibody of claim 38, wherein said antibody is a monoclonal antibody.

40. Method for screening for cancer in a sample, comprising contacting said sample with a nucleic acid molecule which hybridizes to all or part of the molecule encoded by SEQ ID NO: 1, 2, 3, 4, 8, 15, 19, 22 or 26 and determining hybridization as an indication of cancer cells in said sample.

41. A method for screening for cancer in a sample, comprising contacting said sample with the isolated antibody of claim 38, and determining binding of said antibody to a target as an indicator of cancer.

42. Method for diagnosing a cancerous condition in a subject, comprising contacting an immune reactive cell containing sample of said subject to a cell line transfected with the isolated nucleic acid molecule of claim 1, and determining interaction of said transfected cell line with said immunoreactive cell, said interaction being indicative of said cancer condition.

43. A method for determining regression, progression of onset of a cancerous condition comprising monitoring a sample from a patient with said cancerous condition for a parameter selected from the group consisting of (i) a protein encoded by SEQ ID NO: 1, 2, 3, 4, 8, 15, 19, 22 or 26, (ii) a peptide derived from said protein, (iii) cytolytic T cells specific for said peptide and an MHC molecule with which it non-covalently complexes, and (iv) antibodies specific for said CT protein, wherein amount of said parameter is indicative of progression or regression or onset of said cancerous condition.

44. The method of claim 43, wherein said sample is a body fluid or exudate.

45. The method of claim 43, wherein said sample is a tissue.

46. The method of claim 43, comprising contacting said sample with an antibody which specifically binds with said protein or peptide.

47. The method of claim 46, wherein said antibody is labelled with a radioactive label or an enzyme.

48. The method of claim 46, wherein said antibody is a monoclonal antibody.

49. The method of claim 43, comprising amplifying RNA which codes for said protein.

50. The method of claim 49, wherein said amplifying comprises carrying out polymerase chain reaction.

51. The method of claim 42, comprising contacting said sample with a nucleic acid molecule which specifically hybridizes to a nucleic acid molecule which codes for or expresses said protein.

52. The method of claim 49, wherein said nucleic acid molecule comprises SEQ ID NO: 9, 10, 11, 12, 13, 14, 17, 18, 20, 21, 24, 25, 28 or 29.

53. The method of claim 43, comprising assaying said sample for shed protein.

54. The method of claim 43, comprising assaying said sample for antibodies specific for said protein, by contacting said sample with protein.

55. Method for diagnosing a cancerous condition comprising assaying a sample taken from a subject for an immunoreactive cell specific for a peptide derived from a protein encoded by SEQ ID NO: 1, 2, 3, 4, 8, 15, 19, 22 or 26, complexed to an MHC molecule, presence of said immunoreactive cell being indicative of said cancerous condition.

56. Composition comprising at least one peptide consisting of an amino acid sequence of from 8 to 25 amino acids concatenated to each other in the isolated cancer associated antigen of claim 12, and a pharmaceutically acceptable adjuvant.

57. The composition of claim 56, wherein said adjuvant is a saponin, a cytokine, or GM-CSF.

58. The composition of claim 56, comprising a plurality of MHC binding peptides.

59. Composition comprising an expression vector which encodes at least one peptide consisting of an amino acid sequence of from 8 to 25 amino acids concatenated to each other in the isolated cancer associated antigen of claim 12, and pharmaceutically acceptable adjuvant.

60. The composition of claim 59, wherein said expression vector encodes a plurality of peptides.

61. A method for screening for possible presence of a pathological condition, comprising assaying a sample from a patient believed to have a pathological condition for antibodies specific to at least one of the cancer associated antigens encoded by SEQ ID NOS: 1, 2, 3, 4, 8, 15, 19, 22 or 26, presence of said antibodies being indicative of possible presence of said pathological condition.

62. The method of claim 61, wherein said pathological condition is cancer.

63. The method of claim 61, wherein said cancer is melanoma.

64. The method of claim 61, further comprising contacting said sample to purified cancer associated antigen encoded by SEQ ID NO: 1, 3, 4, 8, 15, 19, 22 or 26.

65. A method for screening for possible presence of a pathological condition in a subject, comprising assaying a sample taken from said subject for expression of a nucleic acid molecule, the nucleotide sequence of which comprises SEQ ID NO: 1, 2, 3, 4, 8, 15, 19, 22 or 26, expression of said nucleic acid molecule being indicative of possible presence of said pathological condition.

66. The method of claim 65, wherein said pathological condition is cancer.

67. The method of claim 65, comprising determining expression via polymerase chain reaction.

68. The method of claim 65, comprising determining expression by contacting said sample with at least one of SEQ ID NO: 9, 10, 11, 12, 13, 14, 17, 18, 20, 21, 24, 25, 28 or 29.

69. A method for determining regression, progression of onset of a cancerous condition comprising monitoring a sample from a patient with said cancerous condition for a parameter selected from the group consisting of (i) a cancer associated antigen encoded by SEQ ID NO: 1, 2, 3, 4, 8, 15, 19, 22 or 25, (ii) a peptide derived from said cancer associated antigen,

(iii) cytolytic T cells specific for said peptide and an MHC molecule with which it non-covalently complexes, and (iv) antibodies specific for said cancer associated antigen, wherein amount of said parameter is indicative of progression or regression or onset of said cancerous condition.

70. The method of claim 69, wherein said sample is a body fluid or exudate.
71. The method of claim 69, wherein said sample is a tissue.
72. The method of claim 69, comprising contacting said sample with an antibody which specifically binds with said protein or peptide.
73. The method of claim 72, wherein said antibody is labelled with a radioactive label or an enzyme.
74. The method of claim 72, wherein said antibody is a monoclonal antibody.
75. The method of claim 69, comprising amplifying RNA which codes for said protein.
76. The method of claim 75, wherein said amplifying comprises carrying out polymerase chain reaction.
77. The method of claim 69, comprising contacting said sample with a nucleic acid molecule which specifically hybridizes to a nucleic acid molecule which codes for or expresses said protein.
78. The method of claim 69, comprising assaying said sample for shed cancer associated antigen.
79. The method of claim 69, comprising assaying said sample for antibodies specific for said cancer associated antigen, by contacting said sample with said cancer associated antigen.

80. Method for screening for a cancerous condition comprising assaying a sample taken from a subject for an immunoreactive cell specific for a peptide derived from a cancer associated antigen encoded by SEQ ID NO: 1, 2, 3, 4, 8, 15, 19, 22 or 26, complexed to an MHC molecule, presence of said immunoreactive cell being indicative of said cancerous condition.

81. An isolated nucleic acid molecule consisting of a nucleotide sequence defined by SEQ ID NO: 1, 2, 3, 8, 15, 19, 22 or 26.

82. Isolated nucleic acid molecule the complimentary sequence of which hybridizes, under stringent conditions, to the nucleotide sequence set forth in SEQ ID NO: 4, 5, 8, 15, 19, 22 or 26.

83. An isolated polypeptide comprising at least 9 consecutive amino acids set forth in SEQ ID NO: 5, 7, 16, 19, 23, 27, or 30.

84. The isolated polypeptide of claim 83, comprising at least 9 consecutive amino acids set forth in SEQ ID NO: 23 or 30.

85. The isolated polypeptide of claim 84, comprising at least 9 consecutive amino acids of the amino acid sequence set forth in SEQ ID NO: 23.

86. The isolated polypeptide of claim 85, comprising amino acids 102-111, 904-912 or 1262-1270 of SEQ ID NO: 23.

87. An isolated nucleic acid molecule which encodes the amino acid sequence of SEQ ID NO: 30.

88. An isolated nucleic acid molecule which encodes the isolated polypeptide of claim 86.

89. Expression vector comprising the isolated nucleic acid molecule of claim 88, operably linked to a promoter.

<110> Jager, Dirk
Scanlan, Matthew
Gure, Ali
Jager, Elke
Knuth, Alexander
Old, Lloyd
Chen, Yao-tseng

<120> Isolated Nucleic Acid Molecules Encoding Cancer Associated Antigens,
the Antigens per se, and Uses Thereof

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<140>

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 ctttgtctcc aagccgttcc aaactgagta ccgggagacg acacaaaggg agggcgggtga 180
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 gaagaagaag cgctccaagg ccaaggcgga gcgagaggcg tcccctgccg acctcccat 720
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 35 40 45

Lys Glu Leu Asp Glu Cys Tyr Glu Arg Phe Ser Arg Glu Thr Asp Gly
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 Ala Gln Lys Arg Arg Met Leu His Cys Val Gln Arg Ala Leu Ile Arg
 65 70 75 80
 Ser Gln Glu Leu Gly Asp Glu Lys Ile Gln Ile Val Ser Gln Met Val
 85 90 95
 Glu Leu Val Glu Asn Arg Thr Arg Gln Val Asp Ser His Val Glu Leu
 100 105 110
 Phe Glu Ala Gln Gln Glu Leu Gly Asp Thr Val Gly Asn Ser Gly Lys
 115 120 125
 Val Gly Ala Asp Arg Pro Asn Gly Asp Ala Val Ala Gln Ser Asp Lys
 130 135 140
 Pro Asn Ser Lys Arg Ser Arg Arg Gln Arg Asn Asn Glu Asn Arg Glu
 145 150 155 160
 Asn Ala Ser Ser Asn His Asp His Asp Asp Gly Ala Ser Gly Thr Pro
 165 170 175
 Lys Glu Lys Lys Ala Lys Thr Ser Lys Lys Lys Lys Arg Ser Lys Ala
 180 185 190
 Lys Ala Glu Arg Glu Ala Ser Pro Ala Asp Leu Pro Ile Asp Pro Asn
 195 200 205
 Glu Pro Thr Tyr Cys Leu Cys Asn Gln Val Ser Tyr Gly Glu Met Ile
 210 215 220
 Gly Cys Asp Asn Asp Glu Cys Pro Ile Glu Trp Phe His Phe Ser Cys
 225 230 235 240
 Val Gly Leu Asn His Lys Pro Lys Gly Lys Trp Tyr Cys Pro Lys Cys
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 35 40 45
 Glu Leu Gly Asp Thr Val Gly Asn Ser Gly Lys Val Gly Ala Asp Arg
 50 55 60
 Pro Asn Gly Asp Ala Val Ala Gln Ser Asp Lys Pro Asn Ser Lys Arg
 65 70 75 80
 Ser Arg Arg Gln Arg Asn Asn Glu Asn Arg Glu Asn Ala Ser Ser Asn
 85 90 95
 His Asp His Asp Asp Gly Ala Ser Gly Thr Pro Lys Glu Lys Lys Ala
 100 105 110

Lys Thr Ser Lys Lys Lys Lys Arg Ser Lys Ala Lys Ala Glu Arg Glu
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 Ala Ser Pro Ala Asp Leu Pro Ile Asp Pro Asn Glu Pro Thr Tyr Cys
 130 135 140
 Leu Cys Asn Gln Val Ser Tyr Gly Glu Met Ile Gly Cys Asp Asn Asp
 145 150 155 160
 Glu Cys Pro Ile Glu Trp Phe His Phe Ser Cys Val Gly Leu Asn His
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 Asn Arg
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 Ala Leu Ile Arg Ser Gln Glu Leu Gly Asp Glu Lys Ile Gln Ile Val
 35 40 45
 Ser Gln Met Val Glu Leu Val Glu Asn Arg Thr Arg Gln Val Asp Ser
 50 55 60
 His Val Glu Leu Phe Glu Ala Gln Gln Glu Leu Gly Asp Thr Val Gly
 65 70 75 80
 Asn Ser Gly Lys Val Gly Ala Asp Arg Pro Asn Gly Asp Ala Val Ala
 85 90 95
 Gln Ser Asp Lys Pro Asn Ser Lys Arg Ser Arg Arg Gln Arg Asn Asn
 100 105 110
 Glu Asn Arg Glu Asn Ala Ser Ser Asn His Asp His Asp Asp Gly Ala
 115 120 125
 Ser Gly Thr Pro Lys Glu Lys Lys Ala Lys Thr Ser Lys Lys Lys Lys
 130 135 140
 Arg Ser Lys Ala Lys Ala Glu Arg Glu Ala Ser Pro Ala Asp Leu Pro
 145 150 155 160
 Ile Asp Pro Asn Glu Pro Thr Tyr Cys Leu Cys Asn Gln Val Ser Tyr
 165 170 175
 Gly Glu Met Ile Gly Cys Asp Asn Asp Glu Cys Pro Ile Glu Trp Phe
 180 185 190
 His Phe Ser Cys Val Gly Leu Asn His Lys Pro Lys Gly Lys Trp Tyr
 195 200 205
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 Glu Lys Ser Lys Lys Glu Arg Ala Tyr Asn Arg
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cgtggtcgtg gttgctggac gcg 23

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 35 40 45

Glu Gln Thr Leu Arg Ala Asp Glu Ile Leu Pro Ser Glu Ser Lys Gln
 50 55 60

Lys Asp Tyr Glu Glu Ser Ser Trp Asp Ser Glu Ser Leu Cys Glu Thr
 65 70 75 80

Val Ser Gln Lys Asp Val Cys Leu Pro Lys Ala Thr His Gln Lys Glu
 85 90 95

Ile Asp Lys Ile Asn Gly Lys Leu Glu Glu Ser Pro Asp Asn Asp Gly
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Phe Leu Lys Ala Pro Cys Arg Met Lys Val Ser Ile Pro Thr Lys Ala
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Leu Glu Leu Met Asp Met Gln Thr Phe Lys Ala Glu Pro Pro Glu Lys
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Pro Ser Ala Phe Glu Pro Ala Ile Glu Met Gln Lys Ser Val Pro Asn
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Lys Ala Leu Glu Leu Lys Asn Glu Gln Thr Leu Arg Ala Asp Gln Met
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Phe Pro Ser Glu Ser Lys Gln Lys Lys Val Glu Glu Asn Ser Trp Asp
 180 185 190

Ser Glu Ser Leu Arg Glu Thr Val Ser Gln Lys Asp Val Cys Val Pro
 195 200 205

Lys Ala Thr His Gln Lys Glu Met Asp Lys Ile Ser Gly Lys Leu Glu
 210 215 220

Asp Ser Thr Ser Leu Ser Lys Ile Leu Asp Thr Val His Ser Cys Glu
 225 230 235 240

Arg Ala Arg Glu Leu Gln Lys Asp His Cys Glu Gln Arg Thr Gly Lys
 245 250 255

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 260 265 270

Glu Ala Lys Glu Ile Lys Ser Gln Leu Glu Asn Gln Lys Val Lys Trp

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 Lys Arg Arg Asn Ala Asp Ile Leu Asn Glu Lys Ile Arg Glu Glu Leu
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 340 345 350
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 385 390 395 400
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 Ser Gly Gln Leu Lys Val Leu Ile Ala Glu Asn Thr Met Leu Thr Ser
 435 440 445
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 Leu Thr Arg Gly Trp Gly Arg Ala Trp Pro Trp Lys Gln Ile Leu Lys
 50 55 60
 Glu Leu Asp Glu Cys Tyr Glu Arg Phe Ser Arg Glu Thr Asp Gly Ala
 65 70 75 80
 Gln Lys Arg Arg Met Leu His Cys Val Gln Arg Ala Leu Ile Arg Ser
 85 90 95
 Gln Glu Leu Gly Asp Glu Lys Ile Gln Ile Val Ser Gln Met Val Glu
 100 105 110
 Leu Val Glu Asn Arg Thr Arg Gln Val Asp Ser His Val Glu Leu Phe
 115 120 125
 Glu Ala Gln Gln Glu Leu Gly Asp Thr Val Gly Asn Ser Gly Lys Val
 130 135 140
 Gly Ala Asp Arg Pro Asn Gly Asp Ala Val Ala Gln Ser Asp Lys Pro
 145 150 155 160
 Asn Ser Lys Arg Ser Arg Arg Gln Arg Asn Asn Glu Asn Arg Glu Asn
 165 170 175
 Ala Ser Ser Asn His Asp His Asp Asp Gly Ala Ser Gly Thr Pro Lys
 180 185 190
 Glu Lys Lys Ala Lys Thr Ser Lys Lys Lys Lys Arg Ser Lys Ala Lys
 195 200 205
 Ala Glu Arg Glu Ala Ser Pro Ala Asp Leu Pro Ile Asp Pro Asn Glu
 210 215 220
 Pro Thr Tyr Cys Leu Cys Asn Gln Val Ser Tyr Gly Glu Met Ile Gly
 225 230 235 240
 Cys Asp Asn Asp Glu Cys Pro Ile Glu Trp Phe His Phe Ser Cys Val
 245 250 255
 Gly Leu Asn His Lys Pro Lys Gly Lys Trp Tyr Cys Pro Lys Cys Arg
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<213> Homo sapiens

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 Lys Gln Glu Glu Asp Glu Glu Tyr Ser Cys Asp Ser Arg Ser Leu Phe
 420 425 430
 Glu Ser Ser Ala Lys Ile Gln Val Cys Ile Pro Glu Ser Ile Tyr Gln
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 Lys Val Met Glu Ile Asn Arg Glu Val Glu Glu Pro Pro Lys Lys Pro
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 Ser Ala Phe Lys Pro Ala Ile Glu Met Gln Asn Ser Val Pro Asn Lys
 465 470 475 480
 Ala Phe Glu Leu Lys Asn Glu Gln Thr Leu Arg Ala Asp Pro Met Phe
 485 490 495
 Pro Pro Glu Ser Lys Gln Lys Asp Tyr Glu Glu Asn Ser Trp Asp Ser
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 Ala Thr His Gln Lys Glu Ile Asp Lys Ile Asn Gly Lys Leu Glu Glu
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 Ser Pro Asn Lys Asp Gly Leu Leu Lys Ala Thr Cys Gly Met Lys Val
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 Ala Glu Pro Pro Gly Lys Pro Ser Ala Phe Glu Pro Ala Thr Glu Met
 580 585 590
 Gln Lys Ser Val Pro Asn Lys Ala Leu Glu Leu Lys Asn Glu Gln Thr
 595 600 605
 Trp Arg Ala Asp Glu Ile Leu Pro Ser Glu Ser Lys Gln Lys Asp Tyr
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 Lys Asp Val Cys Leu Pro Lys Ala Ala His Gln Lys Glu Ile Asp Lys
 645 650 655
 Ile Asn Gly Lys Leu Glu Gly Ser Pro Val Lys Asp Gly Leu Leu Lys
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 Met Asp Met Gln Thr Phe Lys Ala Glu Pro Pro Glu Lys Pro Ser Ala
 690 695 700
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 Pro Pro Glu Lys Pro Ser Ala Phe Glu Pro Ala Ile Glu Met Gln Lys
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 Ser Val Pro Asn Lys Ala Leu Glu Leu Lys Asn Glu Gln Thr Leu Arg
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 Gln Glu Glu Glu Lys Arg Arg Asn Ala Asp Ile Leu Asn Glu Lys Ile
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Asn Glu Ser Lys Cys Thr Ala Leu Met Leu Ala Ile Cys Glu Gly Ser
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Ser Glu Ile Val Gly Met Leu Leu Gln Gln Asn Val Asp Val Phe Ala
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Glu Asp Ile His Gly Ile Thr Ala Glu Arg Tyr Ala Ala Ala Arg Gly
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 Leu Lys Leu Lys Gln Lys Thr Val Thr Lys Arg Ala Ser Gln Tyr Arg
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 Leu Lys Glu
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 Arg Pro Ser Pro Phe Ser Gln Leu Val Tyr Thr Ser Asn Asp Ser Tyr
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 35 40 45
 Gly Gln Val Arg Lys Leu Glu Lys
 50 55